

# APPLICATION NOTE

## Zebrafish observation using the Z stacking function

*Using Celloger® Mini Plus*

Zebrafish, along with mice, is a well-known animal model widely used in biological research such as epigenetics<sup>1</sup>, clinical research<sup>2</sup>, neuroscience research<sup>3</sup> and so on. Mainly, this is because it is easy to manipulate the zebrafish embryos and larvae genetically, and they are easy to observe using an optical microscope since they are small in size and have high optical clarity.

We introduce the Celloger® series' functions for observing zebrafish. Celloger® Mini Plus provides a Z-stacking function that automatically generates multi-layer images and the Celloger® analysis software offers a merge function to combine the multi-layered fluorescent images easily. These functions enable researchers to observe three-dimensional (3D) structures in a single image. We observed transgenic zebrafish (larvae) expressing green fluorescent protein (GFP) using these functions, as detailed in steps 1 and 2 below.

### ■ Step1. Z-stacking

The 3D structures, such as tissue composed of cells, provide various information depending on the focal point; it is crucial to identify various focal points. By setting the distance between layers ("step") and the number of images taken ("N, N'"), Celloger® generates multi-layer images at regular intervals ("step") above and below the set position (Z position) (Figure 1). Different settings can be entered for each point, and different Z-stacking can be performed at various points.

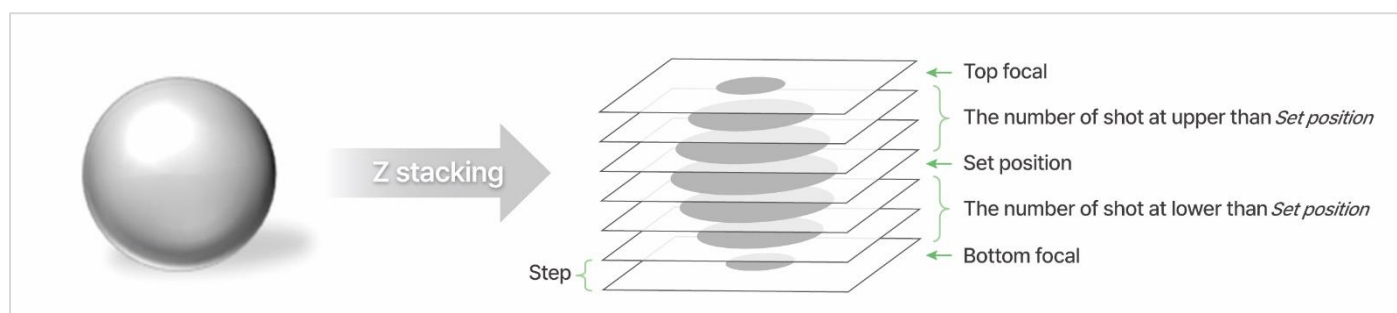


Figure 1. Z stacking

We utilized Celloger® Mini Plus, which enables fluorescence imaging, to create Z-stacked images of the fluorescent transgenic zebrafish. Because the acceptable focus plane varies for each part of the three-dimensional larva, we verified the appropriate focal planes by manipulating the motorized Z stage of the process. We identified the top ( $Z = 4.585$ ) and bottom ( $Z = 4.685$ ) focal planes among several focal planes and the coordinate ( $Z = 4.635$ ) was set corresponding to the middle as the scan position. In addition, we set to take five pictures at  $10\text{ }\mu\text{m}$  intervals above and below the scan position as the center (Figure 2). Figure 3 shows the pictures captured from the top, middle, and bottom focal planes of the zebrafish. In the side view, the shape of the head was definite in the bottom focal plane, while the ventral part was clearly visible in the top focal plane.

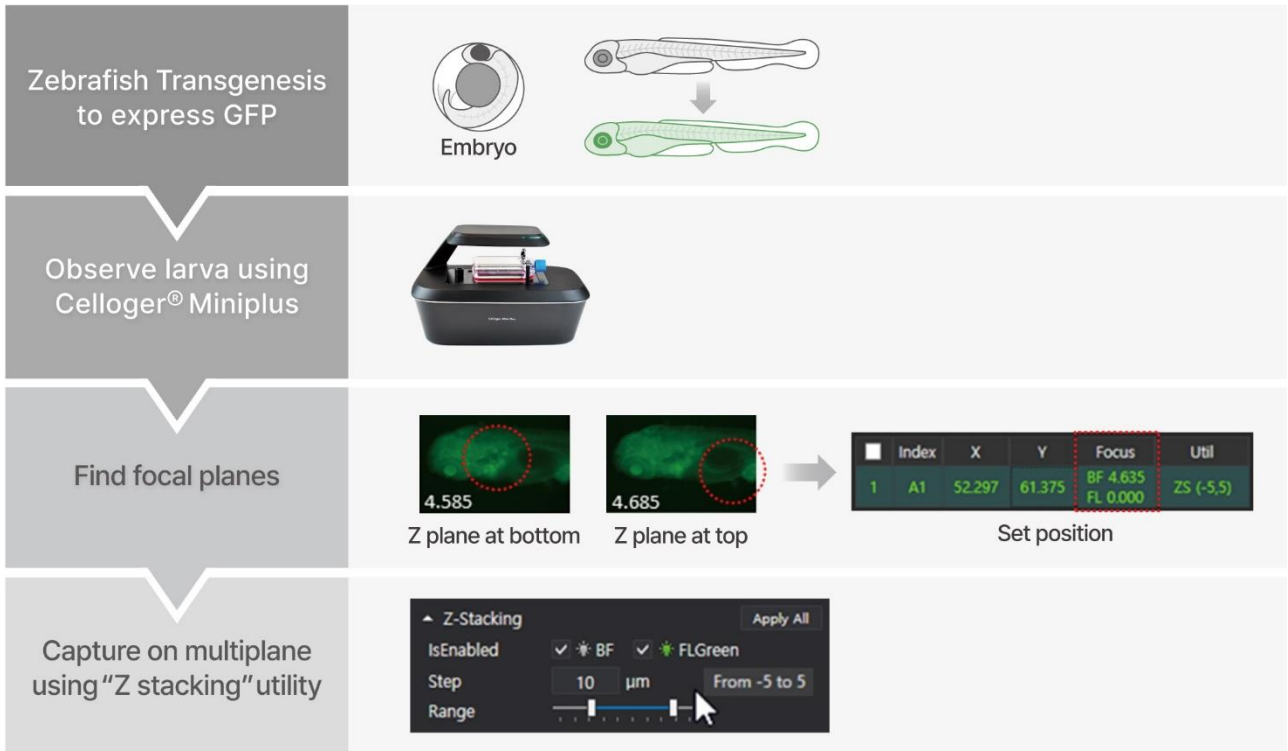


Figure 2. Procedure

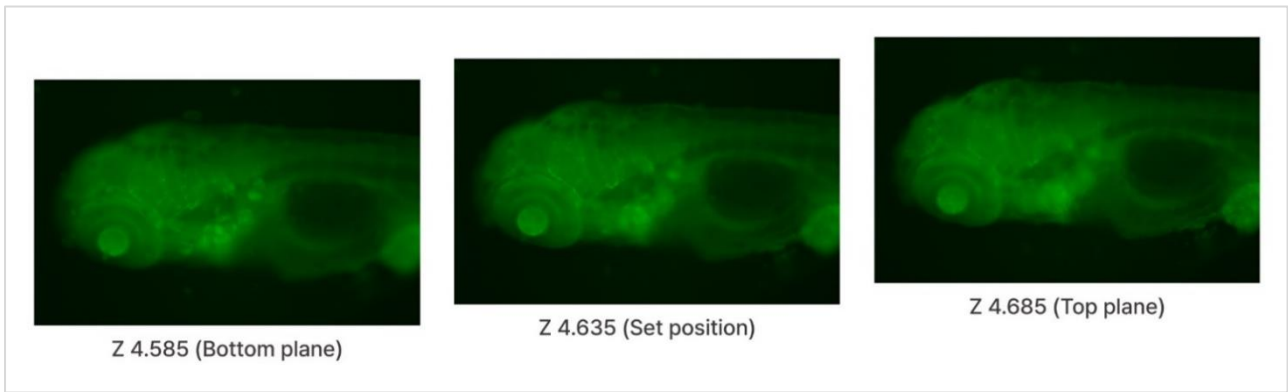
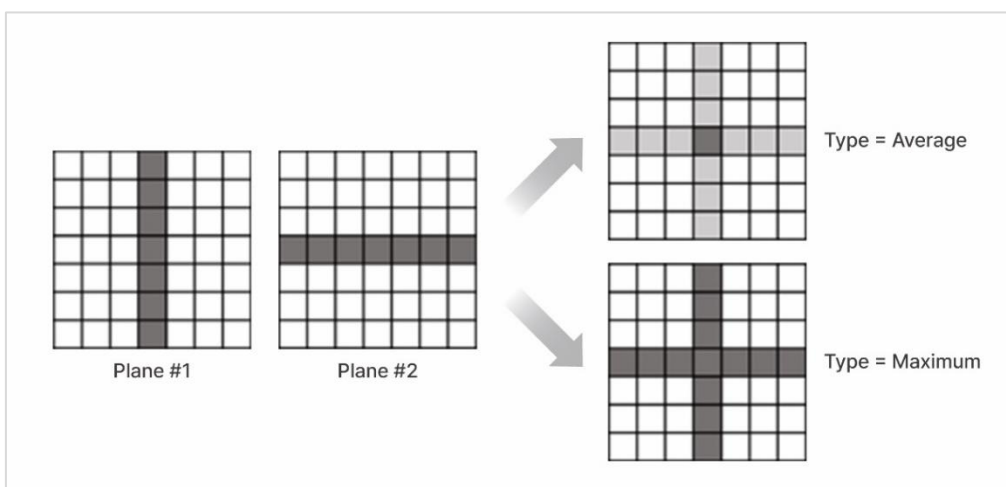


Figure 3. Result

## ■ Step2. Z-projection

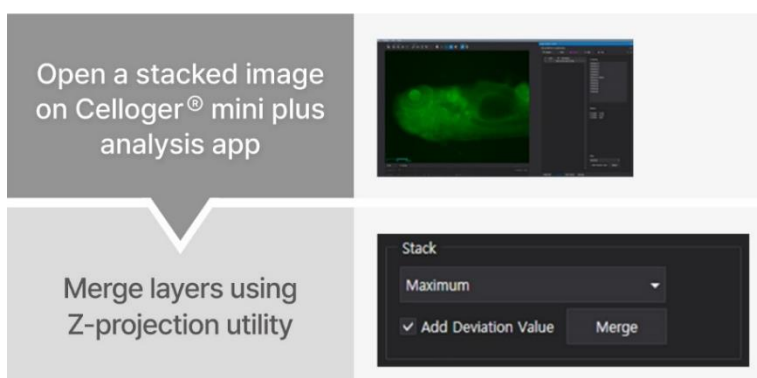
Multi-layer imaging (Z-stacking) is essential for observing the 3D model. However, interpreting the results of multiple images obtained by multi-layer imaging is laborious and time-consuming. The Z-projection function, which merges several layers into one image, allows one 3D sample to be observed at a glance as one image, thereby increasing the researcher's insight.

Projection type is a method of integrating several Z coordinates located at each X-Y position. The "maximum" is used to integrate the brightest pixel among several Z coordinate points, while the "average" is used to calculate the average brightness of several Z coordinate points (Figure 1). In addition, "add deviation value," an element that can be reinforced, was added to the Celloger<sup>®</sup> analysis software to obtain a clearer image.

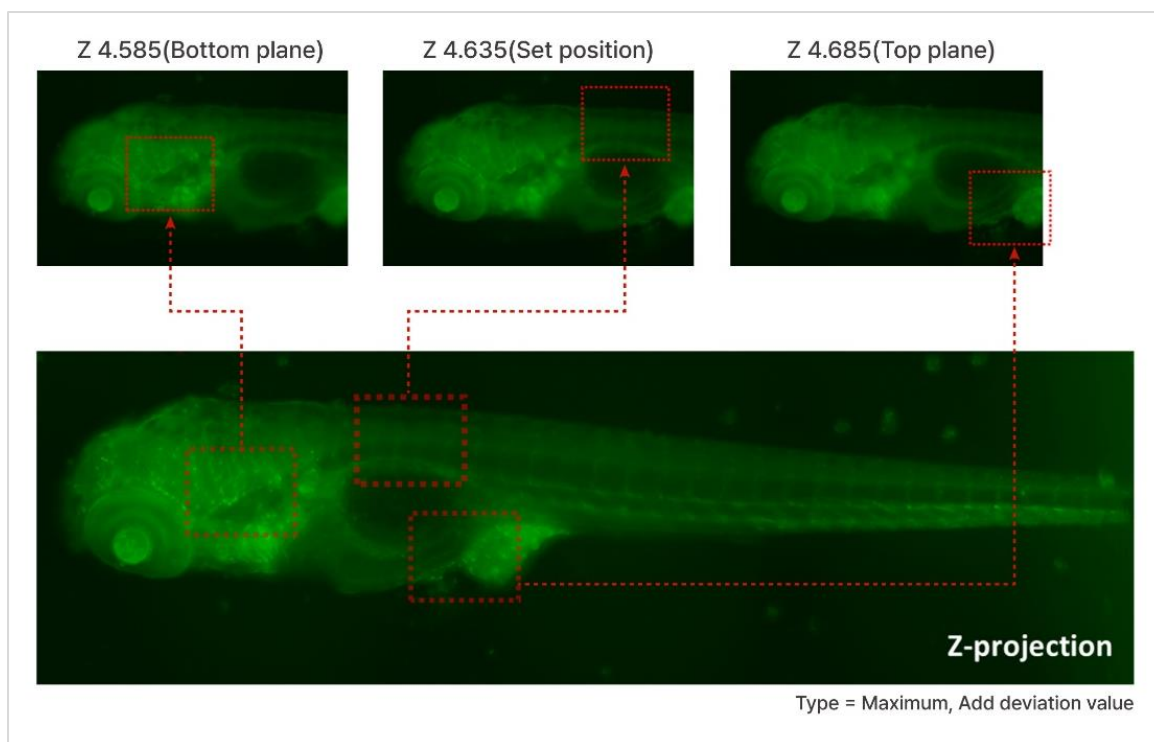


**Figure 1. Explanation of Z projection type**

If the images taken with Z-stacking are opened in the Celloger<sup>®</sup> analysis software, and the "merge" button on the Z-stack tab is clicked, a Z-projection image can be obtained (Figure 2). The appropriate projection type differs among samples; confirming the result for each type is recommended. Figure 3 shows the result of stitching three images for projection by selecting the "maximum" type and "add deviation value" option. The head structure is clearly expressed on the bottom focal plane, and the unique ventral shape, which can be observed on the top focal plane, is expressed as a single image using the projection function.



**Figure 2. Procedure**



**Figure 3. Result**

## ■ Conclusion

We have successfully acquired high-quality fluorescence images of Zebrafish using the Celloger® Mini Plus system. The three-dimensional structure's multiple layers of information were successfully combined and expressed in a single image with clarity. This experiment confirms the feasibility of capturing 3D images using various other sample types, including spheroids and organoids which could expand the range of research scope available to researchers.

## Reference

1. Balasubramanian, S., Raghunath, A., & Perumal, E. (2019). Role of epigenetics in zebrafish development. *Gene*, 718, 144049. <https://doi.org/10.1016/j.gene.2019.144049>
2. Kinth, P., Mahesh, G., & Panwar, Y. (2013). Mapping of zebrafish research: a global outlook. *Zebrafish*, 10(4), 510–517. <https://doi.org/10.1089/zeb.2012.0854>
3. Stewart, A. M., Braubach, O., Spitsbergen, J., Gerlai, R., & Kalueff, A. V. (2014). Zebrafish models for translational neuroscience research: from tank to bedside. *Trends in neurosciences*, 37(5), 264-278.